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Some Concepts in Toxicology

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Toxicology seeks to understand and quantify injurious chemico-biological interactions. The application of this understanding is prediction of the likelihood of occurrence of injury to human health or to undesirable alteration of ecological balance. The key to understanding chemical induced biological injury is development of improved methods of measuring changes in cellular function and structure and the application of these methods to elucidate the mechanisms and factors that modulate chemical injuries. The key to application of this understanding is appropriately designed dose-response and time-response studies which will, with appropriate considerations of biological mechanisms, allow prediction of conditions of exposure (and their confidence limits) that represent finite levels of risk of injury. The underlying data base required is extensive and will be drawn from traditional studies as well as new methods of testing and risk assessment.

Toxicology has recently been defined by the educational committee of the Society of Toxicology as "the science which studies the adverse effects of chemicals on living organisms and assesses the probability of their occurrence" (1). Thus, a satisfactory toxicological assessment of a chemical will include not only the identification, quantitation and interpretation of injurious effects of chemicals in living systems, but it will also include a quantitative analysis of the routes and mechanisms by which injurious chemicals reach the sensitive organisms and sensitive cells within the organism. The earliest but most durable basic concept in toxicology is said to have been annunciated in the sixteenth century by Paracelsus who stated that "all substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy."

The primary objective of toxicological testing is to obtain data on the dose-response characteristics of a chemical. These studies provide the primary data base from which estimates of risk to an identified population of organisms may be determined in connection with specific uses or disposal practices for a specific chemical. The choice and sequence of toxicity tests will depend on the questions or hypotheses that are developed. The nature and sequence of tests used to satisfy requirements of regulatory agencies may differ markedly from those used in an investigation of basic mechanisms of toxic action. Differences in approach will also depend on whether the investigation is initiated to evaluate the toxicity of a chemical prior to its introduction into use, i.e., prospective toxicology, or to confirm in laboratory animals (or under laboratory conditions) an epidemiological association that suggests chemical-induced disease in man, i.e., *retrospective toxicology*. Under ideal conditions prospective toxicology will eliminate the need for retrospective toxicity evaluation.

The purpose of undertaking research and testing of the potentially injurious effects of chemicals on living organisms is not to ban these chemicals, but to characterize the nature of the injuries that might be produced and to determine the limiting quantities and/or durations or frequencies of exposure which result in injury. During the decades of the 1940's and 1950's, the Food, Drug and Cosmetic Act of 1938 and its amendments and the Federal Insecticide, Fungicide and Rodenticide Act of 1947 were the major enabling legislation in the United States which formalized requirements for systematic toxicity testing of chemical substances. The toxicological test data obtained, as required by those regulations, even with the test methods of the 1940's and 1950's, appear to have provided a data base adequate to set limits for food additives and pesticide residues that would protect the general public against injury from those chemicals under normal use conditions. At least we have no known chronic disease states that can be clearly attributed to exposure to these regulated sub-

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stances in food. However, there is nagging doubt that the unexplained etiology of a high proportion of birth defects, cancer and some other chronic diseases may be due to chemical exposures, and that failure to verify these presumed associations in humans are due to the insensitivity of — or a total lack of epidemiological studies. Furthermore, as greater knowledge of biology and highly sensitive methods for measuring biological change have developed, biological effects have been detected with exposures to chemicals at dosages once thought to be without biological activity. This, coupled with the rapid growth of chemical technology which introduces hundreds of new chemicals into commerce yearly and with an increasingly informed and concerned public, has led to the enactment of numerous laws and regulations in many countries. These call for an ever-increasing quantity and quality of laboratory research and test data concerning the potential of chemicals to produce injury in living organisms. Although several of these laws and regulations acknowledge the potential for adverse effects of chemicals in ecosystems, the pressure to develop and validate new toxicological test methods has been heavily concentrated on tests intended to evaluate potential for direct effects of exposure on human health.

Estimates of the numbers of chemicals presently in use which will have to be tested and their hazard to health or environment assessed or reassessed, under laws passed in this decade, vary from the tens of thousands to the millions. The number of new chemical substances that will need to be evaluated each year ranges from the hundreds to thousands. In any event, the task is formidable, and considerable attention by various committees has been given to methods for assigning priorities for risk assessment. In a recent major study by the National Academy of Sciences, both biological impact and dispersal of the chemical into the environment were scored in order to arrive at a priority classification (*2*). Table 1shows factors considered to contribute to the biological impact of a chemical. The first three factors relate primarily to the direct interactions of the chemical with the affected biological system. The last three factors relate primarily to the role of transport and fate of the chemical in the environment. The scores arrived at from consideration of biological impact are then combined with estimates of the use and release of the chemical into the environment to arrive at a priority rating for risk assessment as shown in Table 2.

Of course, this scheme assumes a considerable knowledge of the biological disposition and effects of a substance in order to assign scores to arrive at the priority rating of one to twelve. It is perhaps a larger problem to prioritize chemicals for which there is little or no biological data. This must necessarily be done by analogies which place substances into various chemical classes of varied levels of suspicion.

Some knowledge or reasonable basis for suspicion of biological injury is essential for selecting which chemicals are in greatest need of toxicological assessment. However, once the substances have been given priorities for testing, the basic concepts of toxicology apply. The first principle of toxicology states that the severity and/or the incidence of injury is proportional to the dose or some function of the dose. One must bear in mind that dosage is usually measured as the exposure dose, that is the amount ingested, inhaled, or injected. Another form of dosage which requires extensive data to measure is the target dose, that is, the quantity that actually reaches the site of action (i.e., the receptor dose).

Although it is assumed that the receptor dose will vary as a function of the exposure dose, this may not be a direct or linear proportionality. It may be necessary, therefore, in developing mathematical models of dose-response relationships to bear in mind that a different exponent or function may apply when estimating, for example, the molecules of DNA affected when a model animal ingests a millimole of chemical as compared to when a millimole of that chemical is allowed to react with DNA in a test tube. As a sample population of living organisms is exposed to a range of dosages, various possible dose-response relationships can be envisioned as illustrated in Figure 1.

In the first case, A, either the substance is inert in the test organism or the wrong effect has been measured.

The circumstance in case B, the one most often associated with carcinogenic or mutagenic action, envisions a continuum of increasing frequency of response at any finite increment in dosage of the chemical. The severity of effects or frequency of injury are dose-related and if the dose-response curves are sufficiently well characterized and understood, it is theoretically possible to mathematically estimate the number of organisms that would be affected at dosages below those actually tested and in population samples larger than the experimental sample.

Case C is the form of dose-response curve which is most classical in toxicology and upon which most of the environmental quality standards and limits in effect today have been based. In brief, this form of the dose-response curve indicates that there is some finite exposure dose, below which the rates of the biological protective processes of metabolic detox-

Table 1. Factors contributing to biological impact[a]

Factor	Level of importance[b] (1)	(2)	(3)
Toxicity	High	Medium	Low
Receptor importance	High	Medium	Low
Type of effect	Interference with ecosystem functioning	Chronic at the level of the individual	Acute effects at the level of the individual
Availability to organism	High	Low	
Potential for biomagnification	High	Low	
Stability and persistence	High	Low	

[a]NAS data (2).
[b]Low number indicates high significance.

Table 2. Scheme for classification of chemicals according to biological impact and dispersal[a]

Chemical dispersal	Biological impact[b] High (1)	Medium (2)	Low (3)
(1) Widespread, high release	1	2	3
(2) Widespread, low release	2	4	6
(3) Localized, high release	3	6	9
(4) Localized, low release	4	8	12

[a]NAS data (2).
[b]Low number indicates high priority.

ication, excretion, and injury-repair keep pace with or exceed the rates of exposure, absorption and injury-production. This principle underlies the concept of a toxicological threshold. The concept of threshold is most certainly valid for individuals; that is, each individual living organism has its own threshold. However, because of individual variation in the rates of the several biological processes just cited, there will predictably be a wide range of individual dose-response thresholds. Thus, although the application of the threshold concept to individuals is accepted by most toxicologists, its application to a population of individuals is a controversial issue.

The last illustration, D, is one in which adverse effects are associated with both too little and too much of a substance. In the area of food chemicals, dose-response curves of this type are well known for certain nutrients, vitamins or trace elements. For example, trace optimal quantities of chromium or selenium are required for normal function, but are definitely toxic at high levels. In such cases, the traditional application of a safety-factor to the experimentally determined no adverse-effect level, in order to arrive at an acceptable residue or additive limit, may result in an injury from chemical deficiency rather than from chemical excess.

Irrespective of the nature of the response, whether the experimental assessment involves acute or chronic exposure, or whether it is concerned with an air, water, soil or food contaminant, an industrial chemical, a pesticide or a food additive, or a toxic

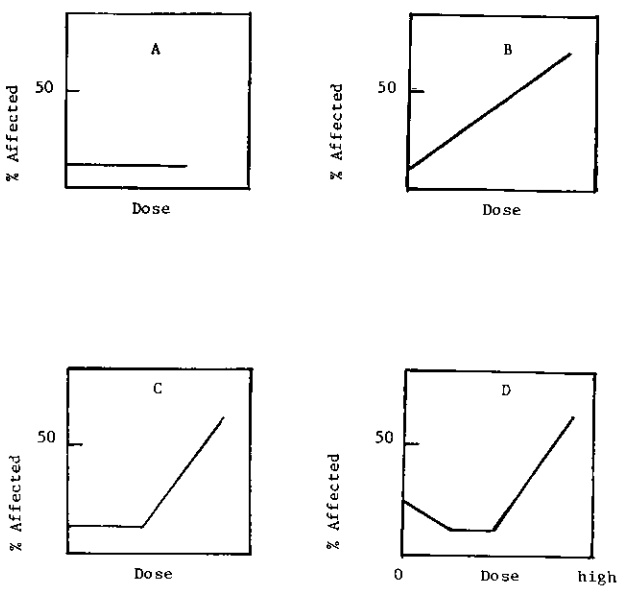

Figure 1. Dose-response possibilities: (A) no effect; (B) no threshold; (C) threshold; (D) low dose beneficial, threshold.

material associated with the production or consumption of energy, the toxicological test data must be obtained with an experimental design that includes a range of exposure doses if these data are to have value in hazard or risk assessment. Ideally some of these doses will fall between the 0 and 100% affected doses so that some estimate of the slope of the dose-response curve can be made.

Traditionally, when data from properly designed experimental protocols are available, toxicologists and other health scientists will reach decisions concerning hazard assessment. Descriptive terms applied to these conclusions have evolved in the toxicological literature. These developed largely out of toxicity assessments for food additives and pesticides, hence "level" refers to level (i.e., concentration) in the diets of test animals. The no-effect level — the term most likely to be found in the older toxicological literature — can be defined as the highest test concentration in the diet of experimental animals that does not result in biological data that differs significantly from animals fed the control diet. Of course, it was only as dependable and comprehensive as the test protocol itself. Furthermore, as biological test methods became more sensitive or as new tests (such as enzyme induction) were added, certain "effects" were detected that did not seem to compromise the test animals' health. This led to the development of additional descriptive terms. Use of the term, "no adverse-effect level" provides an opportunity for scientific judgment concerning the importance of merely a statistically significant effect as compared to an effect that is clearly biologically significant as well.

This allowance for scientific judgment is, on first consideration, salutory. However, there is often controversy as to whether or not an effect is "adverse." For example, some would argue that the induction of liver microsomal enzymes which metabolize foreign organic chemicals (a not infrequent effect of low-dose exposure to organics) is an adaptive response which in itself is not injurious. On the other hand, the fact that some substances are made more, rather than less, biologically active by the action of these inducible enzymes lends credence to the argument that induction of microsomal enzymes must be considered adverse in some cases. It should be recognized, therefore, that use of the term, "no adverse effect," as opposed to simple "no effect," implies that we have sufficient knowledge of the short and long-term consequences of a chemically-induced change in biological structure or function to determine whether that change is injurious, beneficial or of no health consequence. As greater numbers of tests, and, particularly, as more sensitive means for measuring biological changes are included in toxicity assessment protocols, the necessity of distinguishing truly adverse effects will assume even greater importance. Thus, research designed to validate the usefulness of a sensitive biological measurement as a predictive test of injury potential must assume a priority for support equal or greater than research on the development of new methods for measuring biological change.

Increasingly, in recent years the term "no observed adverse-effect level" has been used in place of the simpler, older terminology. Inclusion of the word "observed" merely recognizes the shortcoming of toxicity test protocols and methods that have been used in the case in point. In a sense, it is a subtle acknowledgement of the virtual impossibility of providing the negative and of the inherent weaknesses of any test system based on models.

In spite of these uncertainties and changing attitudes toward the definitiveness of the experimentally derived "no effect" dosage, public health officials and agencies have used these values to form the primary data base from which to estimate the daily dosage that man could ingest throughout a lifetime without appreciable risk to health; that is, the so-called Acceptable Daily Intake (ADI), expressed in mg/kg. Traditionally this has been estimated by dividing the highest measured daily no-effect dosage in experimental studies by a safety factor. The size of this factor varies and generally is inversely proportional to the quantity (and quality) of appropriate data from animal tests and/or human experience. Thus, if data from lifetime exposures are available, the safety factors generally are smaller than if only 3 months exposure data has been generated. Finally, legal limits or guidelines will be promulgated, which may be based upon good manufacturing practice or good agricultural practice, but which, theoretically, would not permit exposure in excess of the ADI.

What is the nature of the data that must be available to arrive at an assessment of health hazard from chemical exposures?

The answer to this, of course, changes (as it should) with time and the development of new knowledge and new test methodology. Furthermore, each chemical substance or proposed use may have characteristics which will require a unique test protocol. Nevertheless the World Health Organization, most national regulatory agencies and the producing industries consider that certain general types of toxicological test data and related information are necessary to form the data base for hazard evaluation of any substance that may reasonably be expected to be regularly ingested (or otherwise contacted) by human populations. These required areas of information and data are: (1) chemical and physical

properties; (2) metabolism and disposition; (3) acute toxicity; (4) repeated short-term exposures; (5) long-term (> ½ lifetime) exposures; (6) special studies.

Knowledge of the chemical and physical properties of a substance is, of course, essential for a number of reasons: to help predict a chemical's distribution in ecosystems and man, to enable development of suitable analytical methods and to enable identification of purity and standardization. The importance of identify and purity of the test substance used in toxicological evaluation should not be underestimated. In recent years, this point has received particular attention in association with the contamination of certain halogenated aromatic compounds with the much more highly toxic halogenated dibenzodioxins and dibenzofurans. The first reports of teratogenicity of the herbicide 2,4,5-T were quite likely due to the presence of as little as 30 ppm of tetrachlorodibenzodioxin arising as a contaminant of the herbicide during the manufacturing process.

The need for establishing chemical identity and standardization applies to the production of technical products to be used in commerce as well as to the toxicological test protocol. Thus, if a product cannot be standardized with respect to its chemical constitution, extrapolation of toxicological data for hazard assessment may be inaccurate since differences in production lots may give rise to difference chemical constitutions which are either more or less toxic than the sample that was experimentally evaluated.

Knowledge of metabolism and disposition of a chemical is useful in predicting sites of injury and likelihood of storage. Metabolic data obtained in both man and animals may aid in the design of detailed and long-term toxicity tests by allowing selection of animal models that most resemble man in their metabolism of the test substance. For some substances that appear in food or water through indirect means, for example pesticide residues, it is also important to know metabolism and distribution of the substance in other living organisms (e.g., both plants and animals) through which the substance passes before it appears as a residue or contaminant in human foods. In the occupational health sphere, it is essential to know the forms of metabolites that may be present in blood or excreted in the urine if biological monitoring of workers, potentially exposed to the parent chemical, is to be meaningful.

Although essentially all hazard assessment protocols call for determination of the LD_{50} of a substance, this statistic, in itself, is usually of limited value with respect to assessing the hazard of a substance in use conditions. It may allow comparison of acute toxicity with other substances, but the relative acute toxicities of two substances, even chemically similar substances, may be quite different than their relative chronic toxicities. Acute toxicity studies are useful in providing data and information relevant to accidental or occupational exposure to relatively large quantities of a substance. Determination of an LD_{50}, if properly conducted by an astute observer, may give some clues as to the nature and sites of injury that may be involved in more chronic exposures, and it is useful in selecting the dosage levels for more extensive chronic studies. Unfortunately, in my opinion, the LD_{50} value has been and still is used too often to place substances into little classification boxes, labeled "highly toxic," "moderately toxic," "practically nontoxic," etc. A substance that fits into the "practically nontoxic" box may, too easily, be dismissed as nonhazardous until years later human experience or an incidental laboratory experiment reveals toxicological properties of a subtle and chronic nature. Vinyl chloride is perhaps the best known recent example to illustrate this point.

Repeated short-term exposures usually involve administration of the substance in the diet, drinking water or inhaled air for a period of several weeks, generally three months in rodents. Properly conducted with a full complement of biological, physiological and morphological assay procedures these studies hold the potential for identifying nearly all types of toxic effects that a substance is capable of producing. Obvious exceptions, of course, are the potential for induction of cancer or heritable mutations, and some allergic phenomena.

Finally, in an attempt to more accurately define the limiting dosages that may result in any injurious effect and to incorporate tests for effects characterized by delayed onset (e.g., carcinogenesis) long-term exposure studies are conducted. These are generally lifetime exposure studies in rodents and for a major fraction of a lifetime in other species.

In addition to the routine clinical evaluation of the test animals' health, determined by biochemical, physiological and morphological assay procedures during the acute, short-term and long-term repeated exposure studies, there are a number of tests commonly referred to as special studies. These include tests for carcinogenesis, mutagenesis, teratogenesis, reproductive effects, delayed neurotoxicity, behavioral effects and potentiation with other chemicals. With today's testing requirements, the term "special studies" is really no longer appropriate, because assays for carcinogenic, mutagenic, teratogenic, and reproductive effects have become a routine of chemical hazard assessment procedures. However, to assess the potential for these "special" effects, it is necessary to design the repeated short and long-term exposure experiments in a manner

that will allow their evaluation. Or, it may be necessary to design a separate experimental protocol specifically for this purpose, for example in testing for mutagenic potential.

Finally, the objective of any toxicity test program is prediction: prediction of biological disposition from physical-chemical constants, prediction of altered cell or organ system function from reaction with macro-molecules, prediction of irreversible consequences of reversible changes, prediction of implications of selected measurable variables to overall health and survival of the test organisms, prediction of effects in individuals of one species from tests conducted in another, and finally prediction of incidence in large populations from tests on small samples. All of these predictions must relate quantitatively to a dose and dose-rate or schedule that can ultimately be related to probably "amounts and manners" of use or occurrence of the chemicals in the environment.

Traditional approaches to toxicity evaluation have generally not attempted to make predictions far removed from the final application or interpretation of the data. Thus, test organisms are exposed to a range of doses and their health status is examined by biochemical, physiological or pathological procedures analogous to those used in clinical medicine. When this approach has been comprehensive, judicious application of the data appears to have been generally successful in preventing chemical-induced disease. Abandoning this approach in favor of new, different or shortcut methods cannot be advocated without thorough verification of their validity. On the other hand, serious consideration must be given to the application of some shorter-term means of predicting toxicity in order to provide a practical means of evaluating the many chemicals already in the environment and those new compounds that are continuously added to the environment and which have not been subjected to "traditional" tests.

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